

EXHIBIT B

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Synthesis of Cht A 176

5' > CGT, TAC, TCG, GAT, GCC, CAA, ATA, TCG, CCA, CAT, TCG <3'

33 BASES TOTAL

Cht A 176

Conditions:

ABI 380A DNA Synthesizer

BCE Phosphoramidite (A from cracker freshly made, G, C, T from cracker med)

Cycle RB3MW

trityl off, manual

G column, ABI, 1.0 mmol (yellow # 320848)

- 33 trityl and color s were collected
column dried \geq Argon gas for 15'

Total weight of resin = 41 mg
 $1/3 = 14$ mg deblocked at 55°C for 3 hours
 27 mg kept at 4°C in small vial

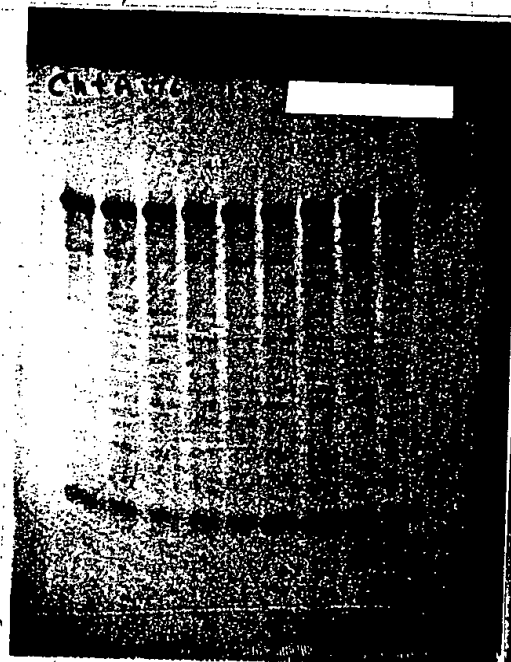
Total Crude O.D. = 69.5
 O.D. gel purified = 27.8 O.D. loaded on a 15%
 denaturing gel in 9 lanes

The slowest moving band was isolated, crushed &
 suspended in 0.1 M NH_4OAc pH = 7.0 O/N at
 Room Temp in wrist shaker.

Cht A 176 desalted using PD-10 column using procedure (237-4). O.D. measured.

Total O.D. Purified = 5.76
 Concentration = 1.28 O.D./ml

Kinaton

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Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

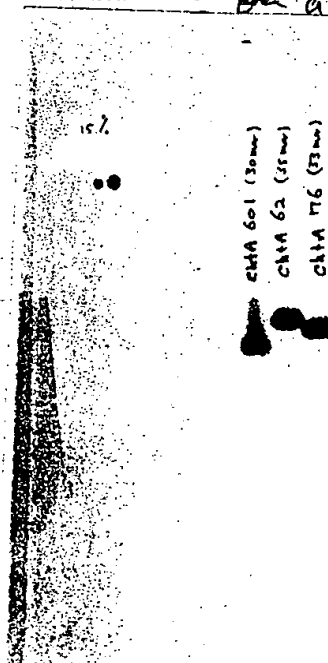
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~~Handwritten note~~

Kination

Base	A498nm	
1 G	1.449	
2 C	1.474	100%
3 T	1.365	93%
4 T	1.358	99%
5 A	1.304	96%
6 C	1.245	95%
7 A	1.238	99%
8 C	1.187	96%
9 C	1.268	100%
10 G	1.226	97%
11 C	1.188	97%
12 T	1.198	100%
13 A	1.172	98%
14 T	1.103	94%
15 A	1.068	97%
16 A	1.057	99%
17 C	0.873	83%
18 C	0.880	100%
19 C	0.867	98%
20 C	0.841	97%
21 G	0.951	>100%
22 T	0.870	91%
23 A	0.842	97%
24 G	0.822	98%
25 G	0.8130	99%
26 C	0.8220	100%
27 T	0.788	96%
28 C	0.781	99%
29 A	0.774	99%
30 T	0.735	95%
31 T	0.733	100%
32 G	0.741	100%
33 C	0.723	97%

7.5 ul CHTA 176
 1.0 ul 10XKB
 1.0 ul γ - 32 P-ATP (G#474)
 0.5 ul T₄ Kinase
 (Pharmacia ND7344)
 Incubate at 37°C for 30'
 72°C for 2'
 Chill on ice
 Precipitate:
 200ul 5M NH₄OAc
 0.5 ul 0.5M EDTA
 600ul glycogen
 800ul ethanol
 Wash in 80% ethanol
 Dissolve in 10ul dH₂O
 Load 2ul (2x10⁵ cpm)
 on a 15% denaturing gel



Also picture of
 autoradiogram
 shows
 CHTA 176 is
 pure & has
 correct length
 of 33 bases
 1 ml = 1.28 OD
 was given
 to the
 requester.

Page No. _____

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[Signature]

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